Cont a1

The present application is a divisional application of U.S. Serial No. 08/663,272 filed on November 25, 1996.--

Please replace the paragraph beginning at Page 3, line 18, with the following rewritten paragraph:

--According to this preferred embodiment, there is provided a recombinant or synthetic peptide or chemical equivalent thereof comprising the sequence: a2

$$X_1X_2X_3$$

wherein

$X_1$ and $X_3$ may be the same or different and each is an amino acid sequence comprising from 0 to 15 naturally or non-naturally occurring amino acid residues; $X_2$ is selected from FFYTPKTRREAED and FWYIPPSLRTLED or a derivative or chemical equivalent thereof and wherein said peptide is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects with pre-clinical or clinical IDDM and determining reactivity by an appropriate assay. Preferred cells include but are not limited PBMCs, anti-coagulated whole blood or tissue biopsy cells and determining reactivity by an appropriate assay.--

Please replace the paragraph beginning at Page 10, line 6, with the following rewritten paragraph:

--Preferably the present invention contemplates a method of assaying the reactivity of a subject to IDDM autoantigen said method comprising contacting a peptide or chemical equivalent thereof comprising the formula a3

$$X_1X_2X_3$$

wherein:

$X_1$ and $X_3$ may be the same or different and each is an amino acid sequence comprising from 0 to 15 naturally or non-naturally occurring amino acid residues; $X_2$ is selected from FFYTPKTRREAED and FWYIPPSLRTLED or a derivative or chemical equivalent thereof and wherein said peptide is capable of reacting with T cells and modifying T-cell function when

incubated with cells from subjects with pre-clinical or clinical IDDM and determining reactivity by an appropriate assay. Preferably cells include but are not limited to peripheral blood mononuclear cells (PBMCs), anticoagulated whole blood and tissue biopsy cells.

Please replace the paragraph beginning at Page 11, line 6, with the following rewritten paragraph:

--BRIEF DESCRIPTION OF THE DRAWINGS--.

Please replace the paragraph beginning at Page 11, line 8, with the following rewritten paragraph:

--Figure 1 shows a comparison of the regions of similarity among mouse and human proinsulins and GADs (SEQ ID NOS:1-7). Similarities are boxed; identities within boxes are shaded. The C-terminus of the mature insulin B-chain and the pro-insulin cleavage site are indicated by the vertical line and arrow respectively.--

Please replace the paragraph beginning at Page 11, line 13, with the following rewritten paragraph:

--Figures 2A and 2B are graphical representations showing the level of cellular proliferation expressed as the delta score following the stimulation of peripheral blood mononuclear cells taken from IDDM at-risk (as described in Example 1) or control subjects with the following peptides in Figure 2A: human GAD65 (residues 506-518); human proinsulin (residues 24-36); irrelevant control peptide; or in Figure 2B tetanus toxoid (CSL Ltd., Melbourne, Australia).--

Please replace the paragraph beginning at Page 14, line 20, with the following rewritten paragraph:

--Blood was drawn from paired IDDM at-risk and HLA-DR matched controls at the same time (within 30 minutes) and processed similarly to reduce the effects of diurnal variation and handling artifacts. Peripheral blood mononuclear cells were isolated from heparinised whole blood by Ficoll-Paque (Pharmacia Biotech) density centrifugation, washed and resuspended in RPMI 1640 medium (Biosciences Pty Ltd) containing 20mM Hepes (CSL Ltd), $10^{-5}$ M 2-mercaptoethanol (BDH), penicillin (100U/ml), streptomycin (100 µg/ml) and 10% v/v autologous

plasma. Aliquots of 200µl ($2x10^5$ cells) were transferred into wells of a 96-well, round-bottomed plate (Falcon) and incubated in replicates of six with the following peptides as final concentrations of 10, 2, and 0.4µg/ml: human GAD65 (506-518), human proinsulin (24-36) (synthesized using an Applied Biosystems Model 431A synthesizer (ABI, Foster City, CA), and an irrelevant control peptide (CRFDPQFALTNIAVRK) SEQ ID NO:8 (Macromolecular Resources, Fort Collins, CO). Tetanus toxoid (CSL Ltd, Melbourne, Australia) at final concentrations of 1.8, 0.18 and 0.018 LfU/ml was used as a positive control. Twelve "autologous only" wells containing cells but without antigen were included as the background control. Plates were incubated at 37°C in a 5% v/v $CO_2$ humidified incubator for 6 days; 0.25µCi of [$^3$H]thymidine (ICN) was added to each well for the last 6 hours. The cells were then harvested onto glass fibre filters and incorporated radioactivity measured by beta-particle counting (Packard Model 2000 Liquid Scintillation Counter). The level of cellular proliferation was expressed as the delta score (DS=mean counts per minute (cpm) incorporated in the presence of antigen, minus the mean cpm of the "autologous only" wells).--

Please replace the paragraph beginning at Page 15, line 25, with the following rewritten paragraph:

--Reactivity to the proinsulin sequence was confined almost entirely to IDDM at-risk subjects, whereas some controls also responded to the GAD peptide (Table 2, Figures 2A and 2B). Both groups responded similarly to tetanus, and no subject reacted to the unrelated control peptide.